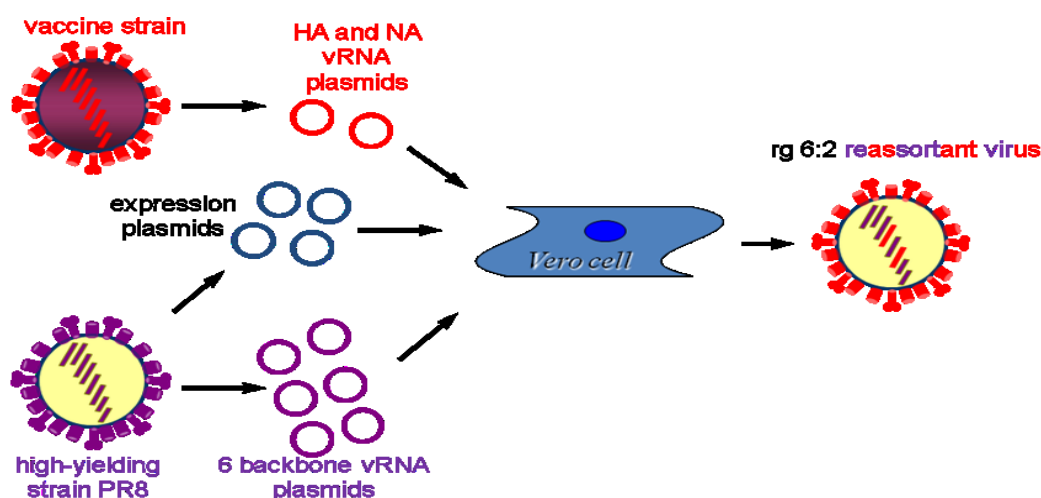


Provenance of the plasmids used by NIBSC to 'rescue' NIBRG viruses

Derivation of NIBRG reference candidate vaccine viruses by reverse genetics

The haemagglutinin (HA) and neuraminidase (NA) genome segments of the wildtype influenza virus are cloned into 'rescue' plasmids. These are transfected into Vero cells together with additional rescue plasmids containing the remaining six viral genome segments from the PR8 virus, and four expression plasmids. Genetically, the rescued reference candidate vaccine virus is a reassortant containing six segments from PR8 and two from the wild type virus.



NIBSC thus uses the 12-plasmid system.

Source of rescue plasmids

The rescue plasmids described herein were received from the University of Oxford, under Material Transfer Agreements.

The first reverse genetics (RG) plasmids received comprised a basic rescue plasmid (pPoll-SapI-Rib), eight rescue plasmids containing each of the eight genome segments of WSN virus in pPoll-SapI-Rib and four expression plasmids capable of expressing the WSN replicase helper function.

The second RG plasmids (received late 2000) comprised a basic rescue plasmid (pPollSapIT), eight rescue plasmids containing each of the eight genome segments of PR8 virus in pPollSapIT and four expression plasmids capable of expressing the PR8 replicase helper function.

NIBSC's understanding is that the rights to the Oxford plasmids passed to the Mount Sinai School of Medicine (MSSM). In 2003, NIBSC requested permission to share with vaccine manufacturers and others reference candidate vaccine viruses made using the plasmids for pandemic preparation purposes. This was agreed under an MTA with MSSM. The terms of this MTA have subsequently been modified by mutual agreement between the parties and NIBSC's understanding is that MSSM has since assigned its interest in this MTA to MedImmune, LLC, a US company.

Use of the rescue plasmids in production of NIBRG viruses

All the reference candidate pandemic vaccine viruses produced at NIBSC were made using the PR8 pPol1SapIT plasmids with WSN helper plasmids. The plasmids used to create each RG virus are the same, with the exception that the HA and NA plasmids have the same pPollSapIT backbone, but different origins of the influenza segments.